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## ARTICLE

# NMR resonance assignments of NarE, a putative ADP-ribosylating toxin from *Neisseria meningitidis*

Ludovic Carlier · Christian Koehler · Daniele Veggi ·  
Mariagrazia Pizza · Marco Soriani · Rolf Boelens ·  
Alexandre M. J. J. Bonvin

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**Abstract** NarE is a 16 kDa protein identified from *Neisseria meningitidis*, one of the bacterial pathogens responsible for meningitis. NarE belongs to the ADP-ribosyltransferase family and catalyses the transfer of ADP-ribose moieties to arginine residues in target protein acceptors. Many pathogenic bacteria utilize ADP-ribosylating toxins to modify and alter essential functions of eukaryotic cells. NarE was proposed to bind iron through a Fe–S center which is supposed to be implied in catalysis. We have produced and purified uniformly labeled  $^{15}\text{N}$ - and  $^{15}\text{N}/^{13}\text{C}$ -NarE and assigned backbone and side-chain resonances using multidimensional heteronuclear NMR spectroscopy. These assignments provide the starting point for the three-dimensional structure determination of NarE and the characterization of the role of the Fe–S center in the catalytic mechanism.

**Keywords** NarE · Pathogenic bacteria · ADP ribosylation · Meningitis · NMR

## Biological context

*Neisseria meningitidis* is a gram-negative bacterium best known for its role in meningitis and septicemia. This human-specific pathogen is considered as a major cause of morbidity and mortality during childhood in industrialized countries and is responsible for epidemics in Africa and in Asia (Genco and Wetzler 2010). Like most bacterial pathogens, *N. meningitidis* synthesizes a number of toxic products believed to target and kill their host cells. Among these toxins, proteins that exert ADP-ribosylation activity represent a large family of potentially lethal enzymes able to modify or disrupt essential functions of eukaryotic cells (Moss and Vaughan 1988). ADP-ribosyltransferases catalyze the transfer of a single ADP-ribosyl group from  $\beta$ -nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) onto specific amino acid residues of host cell proteins with the simultaneous release of nicotinamide (Althaus and Richter 1987). Many bacterial pathogens use ADP-ribosylating enzymes to block protein synthesis or to alter signal transduction by inactivating key target proteins such as GTP-binding proteins (Locht and Keith 1986).

The putative ADP-ribosylating NarE protein from *N. meningitidis* was recently identified on the basis of a profile-based computational approach (Massignani et al. 2003). NarE shares structural features with toxins from *V. cholerae* and *E. coli*, and, like cholera toxin and heat-labile enterotoxins, retains the capacity to ADP-ribosylate arginine and to hydrolyze NAD in ADP-ribose and nicotinamide. Another feature of NarE is its ability to bind iron through an iron–sulfur center (Fe–S) (Del Vecchio et al. 2009). Interestingly, site-directed mutagenesis and enzymatic assays showed that correct assembly of the iron-binding site is essential for transferase but not for hydrolase activity (Del Vecchio et al. 2009), suggesting for the first

L. Carlier · C. Koehler · R. Boelens · A. M. J. J. Bonvin (✉)  
Bijvoet Center for Biomolecular Research, Science Faculty,  
Utrecht University, Padualaan 8, 3584 CH Utrecht,  
The Netherlands  
e-mail: a.m.j.j.bonvin@uu.nl

D. Veggi · M. Pizza · M. Soriani  
Novartis Vaccines and Diagnostics, 53100 Siena, Italy

### Present Address:

L. Carlier  
Laboratoire des Biomolécules, CNRS UMR7203, UPMC  
Université Paris 06, 75005 Paris, France

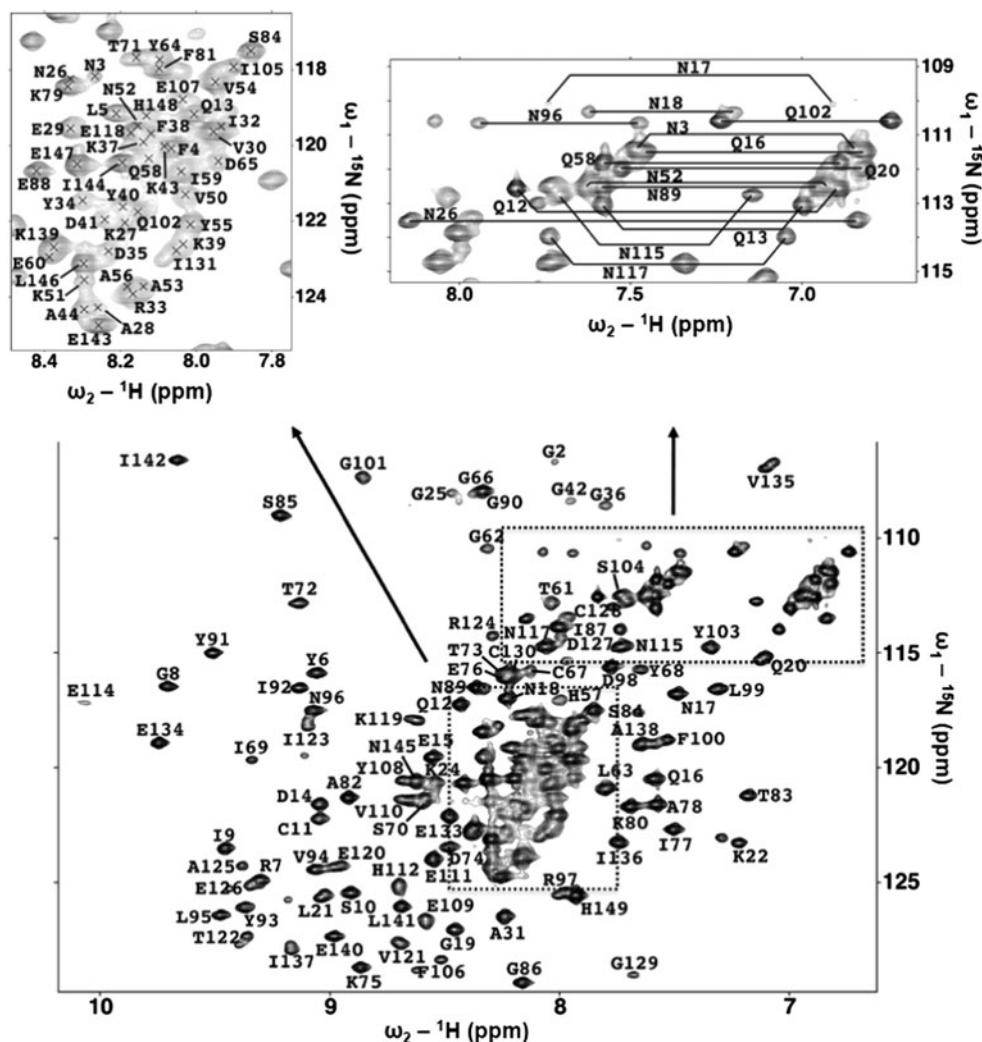
time an implication of a Fe–S cluster in catalysis within the ADP-ribosyltransferase family. We here report the  $^1\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}$  assignments of NarE protein from *N. meningitidis* as a preliminary step towards characterizing its three-dimensional structure and elucidating the role of the iron–sulfur center in the catalytic mechanism.

## Methods and experiments

The *NarE* gene was PCR amplified from the chromosomal DNA of *N. meningitidis* MC58 strain and cloned into a pET21b+ plasmid (Novagen) under the control of the T7 promoter. The resulting expression vector encodes a 17 kDa fusion protein that contains the wild-type NarE sequence (145 residues) followed by a 8-residue C-terminal histidine-tag (LEHHHHHH) used for purification purposes. The pET21b+ plasmid was transformed into an *Escherichia coli* BL21 (DE3) expression strain. Recombinant protein was prepared from cells grown to an  $\text{OD}_{600}$

of 0.5–0.6 at 37°C in a bacterial culture supplemented by 100  $\mu\text{g}/\text{ml}$  ampicillin. Protein expression was then induced by addition of 1 mM isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG), and the culture was further grown for 3 h at 37°C. The cells were harvested by centrifugation ( $8,000\times g$  for 30 min), resuspended in 50 mM phosphate buffer (pH 8.0) containing 300 mM NaCl, and lysed with a French press cell (SLM, Aminco). The soluble fraction enriched with the NarE protein was separated from the inclusion bodies by centrifugation at  $39,200\times g$  for 45 min, and loaded on a nickel affinity chromatography column (GE Healthcare). The protein was purified using IMAC standard protocols and an imidazole concentration of 125 mM was required to elute recombinant NarE. A second step of purification was performed using anion exchange QHP chromatography (GE Healthcare). Fractions containing the target protein were collected at a NaCl concentration of 300 mM and finally concentrated by ultrafiltration using a 3-kDa cutoff membrane (Millipore).

**Fig. 1** Assigned  $^{15}\text{N}$ – $^1\text{H}$  HSQC spectrum of the  $^{15}\text{N}$ -labeled NarE protein acquired at 750 MHz and 27°C. Amide side-chain resonances are connected by solid lines

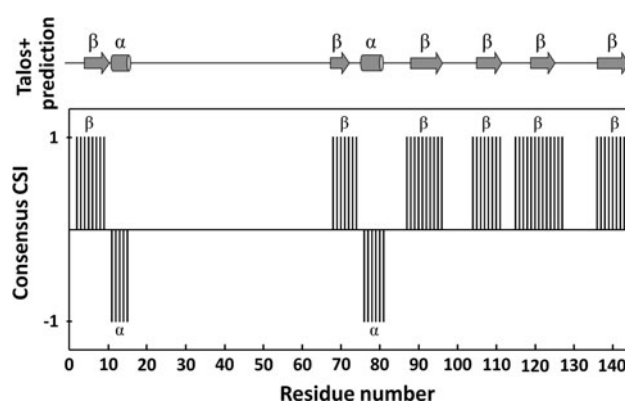


Expression of uniformly labeled  $^{15}\text{N}$  and  $^{15}\text{N}/^{13}\text{C}$  proteins was carried out by growing BL21 strains in ISOGRO- $^{15}\text{N}$  and ISOGRO- $^{15}\text{N}$ ,  $^{13}\text{C}$  rich media (Sigma), respectively. After buffer exchange and concentration, the resulting NMR samples contained approximately 0.6 mM NarE protein in 25 mM phosphate buffer (pH 7.5), 75 mM NaCl, 1 mM PMSF, a protease inhibitor cocktail (Boehringer), 1 mM  $\text{NaN}_3$ , and either 90%  $\text{H}_2\text{O}/10\%$   $\text{D}_2\text{O}$  or 100%  $\text{D}_2\text{O}$ . Trimethylsilyl-[2,2,3,3- $^2\text{H}_4$ ] propionate (TSP) was added as an internal  $^1\text{H}$  chemical shift reference.  $^{13}\text{C}$  and  $^{15}\text{N}$  chemical shifts were referenced indirectly to TSP, using the absolute frequency ratios (Wishart et al. 1995). NMR experiments were performed at  $27^\circ\text{C}$  on a Bruker Avance-II 750 MHz equipped with a triple-resonance ( $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ ) probe. Spectra recorded for sequential backbone assignment were as follows: 2D  $^{15}\text{N}$ - $^1\text{H}$  HSQC, 3D HNCO, 3D HN(CA)CO, 3D HNCA, 3D CBCA(CO)NH, 3D CBCANH, and 3D HBHA(CO)NH. Aliphatic and aromatic  $^1\text{H}$  and  $^{13}\text{C}$  side chain assignments were obtained using 3D HCCH-TOCSY, 3D HCCH-COSY, and 3D (H)CC(CO)NH experiments. All NMR data were processed with the NMRPipe software (Delaglio et al. 1995) and analysed with Sparky (Goddard and Kneller).

### Extent of assignments and data deposition

Figure 1 shows the assigned  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum recorded on the  $^{15}\text{N}$ -labeled protein sample. In this spectrum most of the cross-peaks are well-dispersed, indicating that recombinant NarE is well-folded in the NMR aqueous buffer. All the NH backbone resonances were assigned except for the N-terminal residue M1 and for S49 for which the absence of a cross-peak may be due to loop mobility or conformational exchange as this amino acid follows a proline in the NarE sequence. The lack of  $^{13}\text{C}$  assignments for residue P48 due to line broadening confirms the presence of dynamics in this region of the protein. Interestingly, cysteines C67 and C128, which could be shown to be crucial for the coordination of iron (Del Vecchio et al. 2009), have a  $\text{C}_\beta$  chemical shift value higher than 40 ppm, suggesting the formation of an intramolecular disulfide bridge between them in the absence of a reducing agent. Since the used protein samples were colourless and line broadening of NMR signals due to paramagnetic effects of iron-III could not be observed, the presented resonance assignment likely refers to the protein's apo-form.

Overall, all residues corresponding to NarE were identified: the backbone assignment is complete for  $^1\text{H}_\alpha$  and reaches 98% for  $^1\text{H}_\text{N}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}_\alpha$  and  $^{13}\text{CO}$  nuclei. 88% of the aliphatic side chain resonances were assigned and 94%



**Fig. 2** Secondary structure prediction for the NarE sequence as obtained from TALOS+ and consensus chemical shift index (CSI) programs. The regions identified to adopt  $\alpha$ -helical and  $\beta$ -strand secondary structures are named  $\alpha$  and  $\beta$ , respectively

of the aromatic  $^1\text{H}$  and  $^{13}\text{C}$  resonances were identified including all Phe and Tyr residues. The assignment of  $\text{NH}_2$  side-chain resonances from asparagines and glutamines were also complete except for N145. The chemical shifts have been deposited into the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 16737. The CSI (Wishart and Sykes 1994) and TALOS+ (Shen et al. 2009) programs were used to evaluate NarE secondary structure from  $^1\text{H}_\alpha$ ,  $^{13}\text{C}_\alpha$ ,  $^{13}\text{CO}$  and  $^{13}\text{C}_\beta$  chemical shifts. As shown in Fig. 2, both methods are in agreement with the presence of two  $\alpha$ -helices (residues 11–15, and 76–81) and six  $\beta$ -strands (residues 4–9, 68–72, 88–96, 105–111, 118–125 and 136–143).

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